703. *Polypeptides. Part XI.*[1] *Studies on the Synthesis of Unsymmetrical Peptides of Cystine.*

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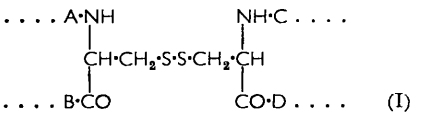
Two routes for the synthesis of unsymmetrical peptides of cystine are outlined. The first involves the coupling of an *N*-protected amino-acid or peptide with an *N*-monosubstituted cystine di-ester, *e.g.*, *N*-monobenzyloxycarbonylcystine diethyl ester; the second involves the coupling of an *N*-protected amino-acid or peptide with a large excess of cystine diethyl ester and further coupling of the unsymmetrical product with another *N*-protected amino-acid or peptide. A number of protected unsymmetrical cystine peptides have been synthesised by these procedures, but their full exploitation awaits the development of more selectively removable *N*-protecting groups.

The development, during recent years, of new and improved methods of peptide synthesis [2] and their successful application to the synthesis of large peptides (*e.g.*, β-corticotropin [3]) makes it possible to envisage the eventual synthesis of true proteins. However, most proteins contain one or more disulphide linkages which join together, as in (I), half-cystine



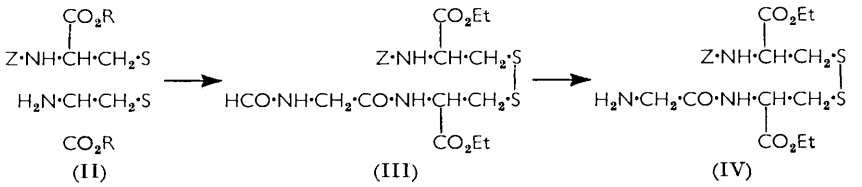
(I)

residues each linked to different amino-acid residues. This feature presents an unsolved synthetic problem, *viz.*, the development of methods for the synthesis of unsymmetrical cystine peptides which might be used as intermediates in the synthesis of structures such as (I). Although small yields of insulin have been obtained [4] by co-oxidation of its two reduced peptide chains, such procedures are of no value for the full confirmation of structures arrived at by degradation. The problem has been discussed by, *inter alia*, Zervas and his colleagues; [5,6] a major difficulty in the synthesis, and synthetic use, of unsymmetrical cystine peptides is the ease with which they undergo disulphide interchange to give mixtures of symmetrical compounds.[7–9] When our work was begun, in 1957, the

[1] Part X, Rydon, *J.*, 1964, 1328.
[2] For a review see Rydon, *Roy. Inst. Chem. Lectures*, 1962, No. 5.
[3] Schwyzer and Sieber, *Nature*, 1963, **199**, 172.
[4] Dixon and Wardlaw, *Nature*, 1960, **188**, 721; Du, Zhang, Lu, and Tsou, *Sci. Sinica*, 1961, **10**, 84; Tsou, Du, and Xü, *ibid.*, p. 332.
[5] Zervas and Photaki, *J. Amer. Chem. Soc.*, 1962, **84**, 3887.
[6] Zervas, Photaki, and Ghelis, *J. Amer. Chem. Soc.*, 1963, **85**, 1337.
[7] Ryle and Sanger, *Biochem. J.*, 1955, **60**, 535; Benesch and Benesch, *J. Amer. Chem. Soc.*, 1958, **80**, 1666.
[8] Schöberl and Gräfje, *Annalen*, 1958, **617**, 71.
[9] Zervas, Benoiton, Weiss, Winitz, and Greenstein, *J. Amer. Chem. Soc.*, 1959, **81**, 1729.

only claim to have prepared unsymmetrical peptides of cystine was the doubtful one of Fischer and Gerngross;[10] since then, three such peptides have been prepared [9,11] and this renewed interest makes it desirable to put on record our own work on this partially solved problem.

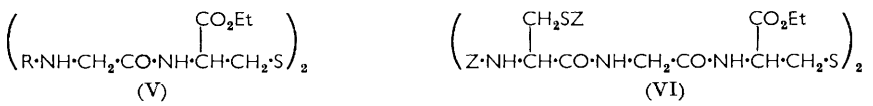
Our first approach involved the use of *N*-monobenzyloxycarbonyl-L-cystine (II; R = H), the preparation [12,13] of which was considerably improved; coupling of the diethyl ester (II; R = Et) with *N*-formylglycine by means of dicyclohexylcarbodi-imide [14] gave the protected unsymmetrical dipeptide (III) in satisfactory yield, as a crystalline solid with a sharp m. p. Treatment of this with cold aqueous alcoholic hydrogen chloride led



(Here and elsewhere Z = $Ph·CH_2·O·CO$.)

to removal of the formyl group, affording the partially protected dipeptide (IV), which seemed to be a promising intermediate for the synthesis of more complex unsymmetrical cystine peptides. Numerous attempts to remove the *N*-benzyloxycarbonyl group from (III) with hydrogen bromide in a variety of solvents (acetic and formic acids, nitromethane) failed owing to disproportionation and we abandoned the method as of insufficient promise; however, the subsequent introduction of more acid sensitive N-protecting groups (*e.g.*, t-butoxycarbonyl [15] and *p*-methoxybenzyloxycarbonyl [16]) makes the method more promising and worthy of further investigation.

Our second, and more successful, approach involved the coupling of an *N*-protected amino-acid with a large excess of L-cystine diethyl ester. In model experiments, directed to the synthesis of symmetrical peptides, two moles of *N*-formyl-, *N*-trityl-, and *N*-benzyloxycarbonyl-glycine were condensed, using dicyclohexylcarbodi-imide, with one of L-cystine diethyl ester to give the protected tripeptides (V; R = HCO, Ph_3C, and Z, respectively); removal of the *N*-protecting groups, to yield the tripeptide ester

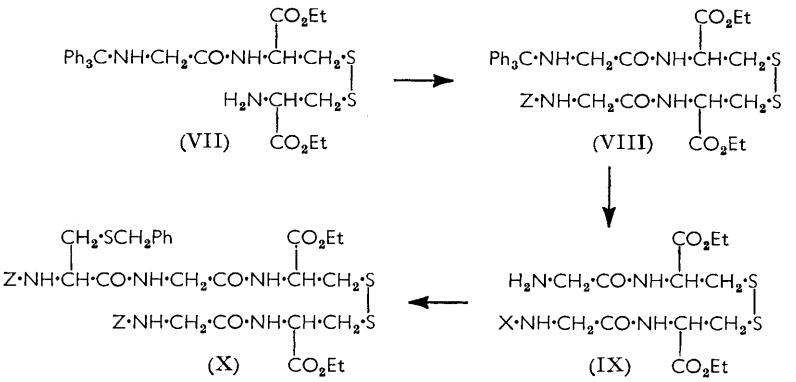


(V; R = H) proceeded most satisfactorily with the trityl compound. Finally, the tripeptide ester (V; R = H) was coupled with *NS*-bisbenzyloxycarbonyl-L-cysteine to give the protected pentapeptide (VI).

In the light of this experience, *N*-tritylglycine was condensed, with the aid of dicyclohexylcarbodi-imide, with a large excess (three moles) of L-cystine diethyl ester. The unsymmetrical partially protected dipeptide (VII) so produced was then further coupled with *N*-benzyloxycarbonylglycine to give the unsymmetrical protected tripeptide (VIII); other protected unsymmetrical tripeptides, analogous to (VIII), were obtained by coupling (VII) with *N*-tritylglycylglycine and *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine. Brief

[10] Fischer and Gerngross, *Ber.*, 1909, **42**, 1485; cf. Abderhalden and Wybert, *ibid.*, 1916, **49**, 2449.
[11] Zahn and Otten, *Annalen*, 1962, **653**, 139; Weygand and Zumach, *Z. Naturforsch.*, 1962, **17**b, 807.
[12] Swan, *Proc. Internat. Wool. Text. Res. Conference*, 1955, C25.
[13] Marshall, Winitz, Birnbaum, and Greenstein, *J. Amer. Chem. Soc.*, 1957, **79**, 4538
[14] Sheehan and Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.
[15] McKay and Albertson, *J. Amer. Chem. Soc.*, 1957, **79**, 4686; Schwyzer, Sieber, and Kappeler, *Helv. Chim. Acta*, 1959, **42**, 2622; Schwyzer and Rittel, *ibid.*, 1961, **44**, 159.
[16] Weygand and Hunger, *Chem. Ber.*, 1962, **95**, 1.

treatment of (VIII) with hot ethanolic hydrogen chloride removed the *N*-trityl without affecting the *N'*-benzyloxycarbonyl group, giving the partially protected tripeptide (IX), with one free α-amino-group; this was finally coupled with *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine to give the protected unsymmetrical tetrapeptide (X). Although the various



unsymmetrical peptides prepared in this way were difficult to purify and had unsharp melting points, possibly owing to disulphide interchange on heating, they were chromatographically homogeneous and clearly not mixtures of symmetrical peptides.

The method, just outlined, for the synthesis of (X) is obviously, in principle, applicable to the synthesis of any protected unsymmetrical cystine peptide carrying different peptide chains attached to the two amino-groups of the cystine residue. In its present form it suffers, like the first method, from the limitations imposed at the time by the lack of a sufficiently wide range of selectively removable *N*-protecting groups [17] but, as with the first method, recent developments [2,15,16] make it, too, promising and worthy of further investigation.

The preparations, by standard methods, of a number of peptides of *NS*-bisbenzyloxycarbonyl-L-cysteine, required for another envisaged procedure, not pursued owing to lack of time, are recorded in the Experimental section.

Experimental

The purity of all products was checked by descending chromatography on Whatman No. 1 paper, using acetic acid : butan-1-ol : water (16·7 : 100 : 37·6 v./v., equilibrated for 30 days at room temp.) (R_{FA}) or pyridine : butan-1-ol : water (21 : 39 : 39 v./v.) (R_{FP}) for development. Spots were detected with ninhydrin, cyanide–nitroprusside [18] or chlorine–starch–iodide.[19]

Evaporations and concentrations were all carried out under reduced pressure.

Synthesis of Peptides from Monobenzyloxycarbonyl-cystine.—*N-Monobenzyloxycarbonyl-L-cystine.* The following modified preparation gave better results than those of earlier workers: [9,12,13]

Benzyl chloroformate (234 g.) was added (30 ml. every 15 min.), with vigorous stirring at 0°, to L-cystine (192 g.) in N-sodium hydroxide (1600 ml.); more sodium hydroxide (200 ml.) was added, in portions, from time to time, to keep the mixture alkaline to phenolphthalein. After further stirring for 2 hr. at 0° and 2 hr. at room temperature, 5N-hydrochloric acid (150 ml.) was added to bring the pH to 5·8. After 1 hr. at 0°, L-cystine (46 g.; 24%) was removed by filtration and washed with water.

The combined filtrate and washings were brought to pH 2·5 with more 5N-hydrochloric acid (95 ml.) and kept at 0° for 4 hr. The precipitate (270 g.; m. p. 184—186°) was collected by

[17] Cf. Schwyzer, " Protides of the Biological Fluids," Elsevier, Amsterdam, 1961, p. 27.
[18] Toennies and Kolb, *Analyt. Chem.*, 1951, **23**, 823.
[19] Rydon and Smith, *Nature*, 1952, **169**, 922.

filtration, washed with water, thoroughly dried in a vacuum desiccator, powdered and extracted with boiling ethyl acetate (1·5 l.) the insoluble residue being collected by filtration and washed copiously with ethanol and ether; *NN'*-bisbenzyloxycarbonyl-L-cystine (87 g.; 25%) was recovered from the extract and washings by evaporation.

The insoluble residue (155 g.; m. p. 188—190°) was suspended in water (3 l.), brought to pH 6·3 with 2N-sodium hydroxide (175 ml.) and stirred at room temperature overnight. L-Cystine (6 g.; 3%) was removed by filtration and the filtrate brought to pH 2·7 with 5N-hydrochloric acid (62 ml.). The precipitate, collected by filtration, washed with water and dried, was the required *N*-monobenzyloxycarbonyl-L-cystine (124 g.; 41%), m. p. 189—191°, R_{FA} 0·33, R_{FP} 0·41, $[\alpha]_D^{20}$ −176° (*c* 1·2 in 0·5N-potassium bicarbonate), −110° (*c* = 1·0 in 5N-hydrochloric acid); earlier workers recorded m. p. 193—194°, $[\alpha]_D^{21}$ −205° (in 0·5N-potassium hydrogen carbonate) [12] and $[\alpha]_D^{23}$ −126° (in 5N-hydrochloric acid) [13] for products which clearly contained substantial amounts of cystine or the bisbenzyloxycarbonyl compound.

N-*Monobenzyloxycarbonyl*-L-*cystine diethyl ester* (II; R = Et). *N*-Monobenzyloxycarbonyl-L-cystine (40 g.) was suspended in anhydrous ethanol (800 ml.) and dry hydrogen chloride passed in until solution was complete (18·0 g. absorbed in 25 min.). The solution was refluxed on a boiling-water bath for 6 hr. and then kept overnight at 0°. The crystals (31 g.) which separated were collected by filtration, a further crop (9 g.) being obtained by concentrating the filtrate. The *hydrochloride* (40 g.; 80%) so obtained had m. p. 154—156° and was chromatographically pure (R_{FA} 0·84; R_{FP} 0·90); the analytical specimen, obtained by recrystallisation from water, had m. p. 158—159°, $[\alpha]_D^{20}$ −60·0° (*c* 0·4 in ethanol) (Found: C, 46·25; H, 5·45; N, 5·65. $C_{18}H_{27}ClN_2O_6S_2$ requires C, 46·3; H, 5·8; N, 6·0%).

N-*Benzyloxycarbonyl*-N'-(N-*formylglycyl*)-L-*cystine diethyl ester* (III). Dicyclohexylcarbodiimide (14·5 g.) was added, in portions, to *N*-formylglycine [20] (12·0 g.) and the above ester hydrochloride (28·0 g.), dissolved in anhydrous chloroform (90 ml.), containing diethylamine (9·0 ml.), the temperature being kept below 40° by external cooling. After 12 hr., dicyclohexylurea (15·2 g.; 96%) was removed by filtration, acetic acid (3 ml.) added and the solution washed with water (3 × 100 ml.). The solution was dried (sodium sulphate), evaporated at below 50° and re-evaporated with ethanol (20 ml.). The residue was dissolved in warm acetone (150 ml.) and kept overnight at 0°. Cautious addition of water (900 ml.) to the filtered solution and recrystallisation of the precipitate (26·0 g.) from ethyl acetate (200 ml.) gave the pure *ester* (20·0 g.; 64%), m. p. 96°, $[\alpha]_D^{23}$ −23·0° (*c* = 3·5 in ethyl acetate), R_{FA} 0·89, R_{FP} 0·90 (Found: C, 48·9; H, 5·7; N, 8·2. $C_{21}H_{29}N_3O_8S_2$ requires C, 48·9; H, 5·7; N, 8·1%).

This ester (5·2 g.) was shaken for an hour with hydrogen bromide (2·8 g.) in acetic acid (40 ml.). The solution was evaporated to dryness at 20—25° and the residue kept for 48 hr. in a vacuum desiccator over phosphorus pentoxide, potassium hydroxide, and paraffin wax. The product was triturated with several portions of anhydrous ether; evaporation of the ether afforded a little *NN'*-bisbenzyloxycarbonyl-L-cystine diethyl ester (R_{FA} 0·89). The insoluble residue (3·3 g.), which solidified, was dissolved in tetrahydrofuran (50 ml.) and treated at 0° with triethylamine (1·1 ml.). Recrystallisation of the precipitate from ethanol gave *NN'*-bis-(*N*-formylglycyl)-L-cystine diethyl ester (650 mg.; 28%), m. p. and mixed m. p. 140—143°, R_{FA} 0·70. Treatment of the ultimate residue with ethanol (7 ml.) gave starting material (1·3 g.; 25%), m. p. and mixed m. p. 91—93°, R_{FP} 0·82.

N-*Benzyloxycarbonyl*-N'-*glycyl*-L-*cystine diethyl ester* (IV). The protected peptide ester (III) (2·6 g.) was kept for 48 hr. in ethanol (70 ml.) and concentrated hydrochloric acid (5·5 ml.). Ether (30 ml.) was then added and the solution kept at 0° for 8 days; the crystals (1·0 g.; 40%), which separated, were collected by filtration, a further crop (0·8 g.; 32%) being obtained by evaporating the filtrate and crystallising the residue from ethyl acetate. Recrystallisation, first from ethyl acetate and then from 0·066N-ethanolic hydrogen chloride, gave the *ester hydrochloride monohydrate*, m. p. 118°, $[\alpha]_D^{20}$ −70·0: (*c* 1·3 in water) (Found: C, 44·3; H, 5·9; N, 7·9; S, 12·2; Cl, 6·65. $C_{20}H_{29}N_3O_7S_2,HCl,H_2O$ requires C, 44·3; H, 5·95; N, 7·75; S, 11·8; Cl, 6·5%).

Synthesis of Peptides from Cystine Diethyl Ester. (a) *Symmetrical Peptides.*—L-Cystine diethyl ester dihydrochloride was best prepared by passing dry hydrogen chloride into a refluxing suspension of L-cystine (100 g.) in ethanol (4·5 l.) until solution was complete (44 hr.). The solution was refluxed for a further 24 hr., concentrated until solid began to separate, treated with ether (5 l.) and kept overnight at 0°. The dihydrochloride (147 g.; 96%), m. p.

[20] Sheehan and Yang, *J. Amer. Chem. Soc.*, 1958, **80**, 1154.

186—188°, was collected by filtration; recrystallisation from ethanol or aqueous acetone raised the m. p. to 190—192°, $[\alpha]_D^{20}$ −48·5° (*c* = 3·5 in water), R_{FA} 0·16, R_{FP} 0·80 (lit.,[11] m. p. 185°; $[\alpha]_D$ −48·0°). This salt (3·7 g.), in chloroform (20 ml.), was treated with triethylamine (2·7 ml.); after 30 min. the solution was washed with water (100 ml.) and then with 0·4M aqueous toluene-*p*-sulphonic acid (250 ml.). Concentration of the latter extract, followed by recrystallisation from ethanol, gave L-*cystine diethyl ester bistoluene*-p-*sulphonate*, m. p. 204—205°, $[\alpha]_D^{20}$ −33·0° (*c* 5·0 in water), R_{FA} 0·53 (Found: N, 4·4. $C_{24}H_{36}N_2O_{10}S_4$ requires N, 4·4%).

NN′-*Bis*-(N-*formylglycyl*)-L-*cystine diethyl ester* (V; R = H·CO). Dicyclohexylcarbodi-imide (4·5 g.) was added to *N*-formylglycine (2·1 g.) and L-cystine diethyl ester dihydrochloride (3·7 g.) in anhydrous chloroform (50 ml.), containing diethylamine (2·0 ml.). After 24 hr., acetic acid (0·5 ml.) was added and the mixture filtered; the filtrate was washed with warm water (3 × 80 ml.) and the washings evaporated. The residue was extracted with boiling tetrahydrofuran (200 ml.) and the extract concentrated, affording the *tripeptide* (2·4 g.; 52%), m. p. 143—144° (after recrystallisation from tetrahydrofuran), $[\alpha]_D^{20}$ −99·0° (*c* 2·9 in water), R_{FA} 0·70, R_{FP} 0·80 (Found: C, 41·05; H, 5·5; N, 11·65. $C_{16}H_{26}N_4O_8S_2$ requires C, 41·2; H, 5·6; N, 12·0%).

NN′-*Bis*-(N-*tritylglycyl*)-L-*cystine diethyl ester* (V; R = Ph_3C). Dicyclohexylcarbodi-imide (4·2 g.) was added to *N*-tritylglycine [21] (6·2 g.) and L-cystine diethyl ester dihydrochloride (3·7 g.) in anhydrous chloroform (30 ml.) containing triethylamine (2·7 ml.). Next day the solution was filtered and the filtrate washed successively with water (200 ml.), saturated aqueous sodium hydrogen carbonate (100 ml.) and water (100 ml.), dried over magnesium sulphate, evaporated and re-evaporated with ethanol (10 ml.). The residue was dissolved in ethyl acetate (10 ml.) and the solution filtered after 18 hr. at 0° and evaporated. Trituration of the residue with ether and recrystallisation from ethanol gave the *tripeptide* (6·0 g.; 67%) m. p. 156—157°, $[\alpha]_D^{20}$ −22·6° (*c* 1·5 in acetone), R_{FA} 0·95, R_{FP} 0·96 (Found: C, 69·9; H, 6·0; N, 6·3. $C_{52}H_{54}N_4O_6S_2$ requires C, 69·8; H, 6·1; N, 6·25%).

NN′-*Bis*-(N-*benzyloxycarbonylglycyl*)-L-*cystine diethyl ester* (V; R = Z), prepared similarly, from N-benzyloxycarbonylglycine [22] in 59% yield and recrystallised from ethanol, had m. p. 69—70°, $[\alpha]_D^{20}$ +13·6° (*c* 3·9 in ethyl acetate), R_{FA} 0·93, R_{FP} 0·92 (Found: C, 53·15; H, 5·6; N, 8·55. $C_{30}H_{38}N_4O_{10}S_2$ requires C, 53·1; H, 5·6; N, 8·25%).

NN′-*Bisglycyl*-L-*cystine diethyl ester* (V; R = H). (i) The bisformyl compound (V; R = H·CO) (560 mg.) was stirred with 1M-ethanolic hydrogen chloride (10 ml.) at 40—45° for 30 min. and the mixture kept overnight at room temperature. Filtration, trituration with ether, and recrystallisation from ethanol yielded the *tripeptide dihydrochloride* (300 mg.; 52%), m. p. 170—175°, R_{FA} 0·24, R_{FP} 0·55 (Found: C, 34·1; H, 6·35; N, 10·8. $C_{14}H_{28}Cl_2N_4O_6S_2$ requires C, 34·8; H, 5·85; N, 11·6%).

(ii) The same compound was obtained, in 85% yield, by heating the bistrityl compound (V: R = Ph_3C), with 1M-ethanolic hydrogen chloride at 80° for 3 min.

NN′-*Bis*-(NS-*bisbenzyloxycarbonyl*-L-*cysteinylglycyl*)-L-*cystine diethyl ester* (VI). Dicyclohexylcarbodi-imide (4·5 g.) was added to the above hydrochloride (4·8 g.) and *NS*-bisbenzyloxycarbonyl-L-cysteine [23] (8·0 g.) in anhydrous chloroform (100 ml.), containing triethylamine (2·7 ml.), and the mixture shaken mechanically overnight. Next day the mixture was evaporated to dryness and the residue extracted with boiling ether and with warm water (250 ml.). The residue was dissolved in warm ethyl acetate (30 ml.) and filtered while still warm; addition of ether to the filtrate precipitated a solid which was recrystallised from tetrahydrofuran; the *pentapeptide* (7·5 g.; 65%), after further recrystallisation from ethanol and from acetic acid, had m. p. 150—152°, $[\alpha]_D^{20}$ −78·0° (*c* 3·8 in dimethylformamide), R_{FA} 0·97 (Found: C, 54·8; H, 5·4; N, 7·0; S, 10·7. $C_{52}H_{60}N_6O_{16}S_4$ requires C, 54·15; H, 5·25; N, 7·3; S, 11·1%).

(b) *Unsymmetrical Peptides.*—N-(N-*Tritylglycyl*)-L-*cystine diethyl ester* (VII). Dicyclohexylcarbodi-imide (4·5 g.; 0·022 mole) was added to *N*-tritylglycine (6·2 g.; 0·02 mole) and L-cystine diethyl ester dihydrochloride (22·2 g.; 0·06 mole) in anhydrous chloroform (100 ml.), containing triethylamine (15·0 ml.). After 2 hr. at room temperature the solution was filtered to remove dicyclohexylurea and the filtrate washed successively with water (200 ml.), 0·4M-aqueous

[21] Velluz and Amiard, *Bull. Soc. chim. France*, 1955, 191.
[22] Bergmann and Zervas, *Ber.*, 1932, **65**, 1192.
[23] Berger, Noguchi, and Katchalski, *J. Amer. Chem. Soc.*, 1956, **78**, 4483.

toluene-*p*-sulphonic acid (500 ml.),* water (200 ml.), saturated aqueous sodium hydrogen carbonate (200 ml.), and water (200 ml.), and dried for 10 min. over magnesium sulphate. This solution, which showed only one spot (R_{FA} 0·70) on paper chromatography, was used as such for further couplings.

In one experiment the chloroform solution was dried and evaporated after the third washing. The residue was triturated with ether and dissolved in warm ethyl acetate (35 ml.); on cooling, dicyclohexylurea (0·8 g.) separated and was removed by filtration. Re-evaporation and repetition of the procedure with acetone (10 ml.) removed a little more urea. Trituration of the residue obtained on evaporating the acetone with warm water gave the *peptide toluene-p-sulphonate monohydrate*, m. p. 65—72°, R_{FA} 0·80, R_{FP} 0·75 (Found: C, 57·8; H, 6·4; N, 5·5; S, 12·1. $C_{38}H_{45}N_3O_8S_3,H_2O$ requires C, 58·1; H, 6·0; N, 5·3; S, 12·2%), which resisted all attempts at recrystallisation.

N-(N-*Benzyloxycarbonylglycyl*)-N′-(N-*tritylglycyl*)-L-*cystine diethyl ester* (VIII). Dicyclohexylcarbodi-imide (4·2 g.) was added to *N*-benzyloxycarbonylglycine (4·2 g.) and *N*-(*N*-tritylglycyl)-L-cystine diethyl ester (0·02 mole) in anhydrous chloroform (100 ml.). After 3 hr., dicyclohexylurea (4·3 g.; 96%) was removed by filtration; the filtrate was washed with saturated aqueous sodium hydrogencarbonate, dried over magnesium sulphate, evaporated to dryness, and re-evaporated with ethanol (15 ml.). The residue was dissolved in warm ethyl acetate (25 ml.) and a little more urea removed by filtration after 4 hr. at 0°. Evaporation gave the *protected tripeptide* (9·4 g.; 60%) which, after re-precipitation from dimethylformamide with water, had m. p. 93—96°, R_{FA} 0·89 (Found: C, 62·3; H, 6·5; N, 7·3; S, 8·3. $C_{41}H_{46}N_4O_8S_2$ requires C, 62·6; H, 5·9; N, 7·1; S, 8·15%).

N-(N-*Trityldiglycyl*)-N′-(N-*tritylglycyl*)-L-*cystine diethyl ester*, m. p. 122—125°, R_{FA} 0·96 (Found: C, 68·5; H, 6·1; N, 7·6. $C_{54}H_{57}N_5O_7S_2$ requires C, 68·1; H, 6·0; N, 7·4%) and N-(S-*benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*)-N′-(N-*tritylglycyl*)-L-*cystine diethyl ester*, m. p. 70—75°, $[\alpha]_D^{20}$ +14·2° (*c* 5·3 in chloroform), R_{FA} 0·89 (Found: C, 63·7; H, 6·3; N, 6·4; S, 9·95. $C_{49}H_{54}N_4O_8S_3$ requires C, 63·7; H, 5·9; N, 6·1; S, 10·4%), were prepared similarly from *N*-tritylglycylglycine [24] and *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine,[25] respectively.

N-(S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinylglycyl*)-N′-(N-*benzyloxycarbonylglycyl*)-L-*cystine diethyl ester* (X). The protected tripeptide (VIII) (2·0 g.) was refluxed for 2·5 min. with 1M-ethanolic hydrogen chloride (3 ml.). Evaporation, followed by trituration with warm ether (100 ml.), which removed triphenylcarbinol (0·5 g.; 78%), re-evaporation and trituration with ether at −20° gave a gum; this, on dissolution in acetone, filtration, evaporation, and precipitation from ethanol with ether gave a hygroscopic powder (1·4 g.; 59%), R_{FA} 0·61.

This hydrochloride (2·9 g.) and *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine (2·0 g.) in anhydrous chloroform (15 ml.), containing triethylamine (0·4 ml.) were treated with dicyclohexylcarbodi-imide (1·2 g.). After 12 hr., the mixture was worked up in the usual manner to give the *protected tetrapeptide* (3·0 g.; 40%), m. p. 112—115° (after recrystallisation from benzene), $[\alpha]_D^{20}$ −16·0° (*c* 0·87 in ethanol), R_{FA} 0·81 (Found: C, 55·55; H, 5·9; N, 8·2; S, 11·15. $C_{40}H_{49}N_5O_{11}S_3$ requires C, 55·1; H, 5·65; N, 8·0; S, 11·05%); paper chromatography of a total acid hydrolysate showed the presence of glycine, cystine, and *S*-benzylcysteine.

Peptides of NS-*Bisbenzyloxycarbonylcysteine.*—NS-*Bisbenzyloxycarbonyl*-L-*cysteinylglycine.* Dicyclohexylcarbodi-imide (20 g.) was added to *NS*-bisbenzyloxycarbonyl-L-cysteine (29·2 g.) and glycine ethyl ester (10·3 g.) in chloroform (65 ml.). After 48 hr., the product was worked up as usual; recrystallisation from aqueous acetone gave the *dipeptide ethyl ester* (28 g.; 79%), m. p. 98—100°, $[\alpha]_D^{20}$ −16·5° (*c* = 1·8 in chloroform), R_{FA} 0·85, R_{FP} 0·90 (Found: C, 58·0; H, 5·6; N, 6·7. $C_{23}H_{26}N_2O_7S$ requires C, 58·2; H, 5·5; N, 5·9%). This ester (4·8 g.) was refluxed for 2 hr. with concentrated hydrochloric acid (15 ml.), acetone (50 ml.), and water (35 ml.). The acetone was removed under reduced pressure and the residue extracted with ethyl acetate. Evaporation of the dried extract, followed by trituration with light petroleum (b. p. 40—60°) and recrystallisation from benzene, gave the *dipeptide* (4·0 g.; 90%), m. p. 66—68° $[\alpha]_D^{20}$ −12·5° (*c* 3·2 in chloroform), R_{FA} 0·80 (Found: C, 56·0; H, 5·1; N, 6·7. $C_{21}H_{22}N_2O_7S$ requires C, 56·5; H, 5·0; N, 6·3%).

* This removes the excess of cystine diethyl ester which can be recovered, as its toluene-*p*-sulphonate, by evaporation of the wash liquor; the unsymmetrical peptide remains in the chloroform as its toluene-*p*-sulphonate.

[24] Fischer and Fourneau, *Ber.*, 1901, **34**, 2868.
[25] Harington and Mead, *Biochem. J.*, 1936, **30**, 1598.

Similar condensations of *NS*-bisbenzyloxycarbonyl-L-cysteine, with L-leucine methyl ester, L-tyrosine methyl ester, and glycyl-glycine ethyl ester, respectively, gave: NS-*bisbenzyloxycarbonyl-L-cysteinyl-L-leucine methyl ester* (75% yield), m. p. 93—95° (from methanol), $[\alpha]_D^{20}$ −17·2° (*c* 3·1 in chloroform), R_{FP} 0·80 (Found: C, 60·45; H, 6·25; N, 6·0. $C_{26}H_{32}N_2O_7S$ requires C, 60·45; H, 6·2; N, 5·4%); NS-*bisbenzyloxycarbonyl-L-cysteinyl-L-tyrosine methyl ester* (80% yield), m. p. 82—84° (from benzene), $[\alpha]_D^{20}$ +11·5° (*c* 4·0 in chloroform), R_{FA} 0·91, R_{FP} 0·93 (Found: C, 61·8; H, 5·7; N, 5·8. $C_{29}H_{30}N_2O_8S$ requires C, 61·5; H, 5·3; N, 4·9%); and NS-*bisbenzyloxycarbonyl-L-cysteinylglycylglycine ethyl ester* (65% yield), m. p. 99—101° (from tetrahydrofuran), $[\alpha]_D^{20}$ −13·2° (*c* 9·5 in chloroform), R_{FA} 0·90 (Found: C, 56·6; H, 5·3; N, 8·4. $C_{25}H_{29}N_3O_8S$ requires C, 56·5; H, 5·5; N, 7·9%).

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